



# Clinical utility of serum folate measurement in tertiary care patients: Argument for revising reference range for serum folate from 3.0 ng/mL to 13.0 ng/mL

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## ABSTRACT

**Objective:** Assess the need for folate testing, frequency of corrective action, and determine reference level for serum folate.

**Methods:** Serum folate levels in 5313 samples from 4448 patients, and clinical data were reviewed for patient characteristics and for (a) evidence of corrective action in patients with serum folate values < 5.5 ng/mL, and (b) differences in patients with serum folate levels < 5.5 ng/mL and patients with levels > 25.7 ng/mL.

**Results:** The prevalence of serum folate levels, in patients, < 3.0, < 4.0, < 5.5, < 7.0 and < 13.0 ng/mL was 0.58%, 1.55%, 4.9%, 9.98% and 43.21% respectively. Patients with serum folate levels < 5.5 ng/mL had lower serum albumin and hemoglobin. In 64% of patients with serum folate > 25.7 ng/mL the sample was collected after supplementation with folic acid. Of the 128 patients with serum folate < 5.5 ng/mL documentation of supplementation was present in only 38.9%.

**Conclusions:** Serum folate levels are below the current “normal” level of 3.0 ng/mL in a larger proportion of tertiary care patients than that reported for ambulatory patients. In patients with folate deficiency, corrective action is lacking in > 60% of the patients. Since serum folate levels ≥ 13.0 ng/mL are needed for optimal prevention of neural tube defects in the embryo/fetus, we propose that normal serum folate level should be designated to be ≥ 13.0 ng/mL.

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## 1. Introduction

Malnutrition is present widely, even in the US and other affluent countries, when one considers both over-nutrition, i.e. obesity, and under-nutrition of essential nutrients, such as vitamins and minerals, with deficiency of vitamin D being perhaps the commonest [1–5]. Folate, also called vitamin B9, (called vitamin B11 in some countries) is one of the essential vitamins and is needed for optimal hematopoiesis, neurologic functions and embryonic development of the central nervous system. Along with vitamin B12, it is instrumental in metabolic reactions for synthesis of methionine and nucleic acids [6–8]. Epidemiological studies have documented the role of folate in preventing neural tube defects and this finding was the impetus for supplementing some food stuffs and flour with folic acid, in the USA, starting in 1998. Since the start of supplementation of food stuffs with folic acid, the prevalence of folate deficiency has dropped to < 1% in pregnant women [2,3]. However, with greater recognition of the role of folate in various physiological processes there has been suggestion that folate sufficiency needs to be redefined [7,9–11].

There is considerable variation in normal or reference value of serum folate, as presented in the literature [6,7,9–14]. In the case of folate it is only the lower limits of folate concentration that is relevant as there are no known ill-effects from high levels of folate and a state of folate hyper-vitaminosis has not been documented. The only potential risk of folate supplementation being that neurologic damage due to concurrent vitamin B12 deficiency may be exacerbated. Depending on the source of information the lower limit of normal

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serum folate value ranges from 2.0 to 7.0 ng/mL [1 ng/mL=2.266 nmol/L] [4,7–19]. Generally levels below 3.0 ng/mL are considered a state of deficiency and levels below 4.0 ng/mL are considered insufficient. These levels are based, in part, on the NHANES data [13]. Serum folate levels of 3.0 ng/mL or greater are sufficient for avoiding megaloblastic anemia. Hyperhomocysteinemia is prevented with serum folate level of  $\geq 4.0$  ng/mL, thus accounting for some sources using a level of 4.0 ng/mL or greater as normal [7,13,16,17]. However, even using the prevention of elevated serum level of homocysteine may require a serum folate level of about 7.0 ng/mL for an optimal effect [18–20]. Many hospitals and a large reference laboratory use serum folate levels of  $< 3.4$  ng/mL as indicative of deficiency and  $< 5.5$  ng/mL as evidence of folate insufficiency [21].

It is noteworthy that supplementation of food stuffs was instituted for the prevention of neural tube defects (NTD) in the embryo/fetus. Studies revealed that red cell folate levels of  $> 906$  and  $> 1292$  nmol/L were needed to obtain optimal benefit of NTD prevention [9–11,22–24]. The consensus, optimum red cell folate level has been designated to be 1000 nmol/L. This blood level of folate has been translated to 7.0 ng/mL by some [10]. However, a more liberal interpretation of red cell folate level of 1000 nmol/L leads us to translate it to serum folate level of 13.0 ng/mL, according to the following calculation from the data in WHO literature: Normal serum and red cell folate levels were listed as 10 and 340 nmol/L [14]. Converting red cell folate level of 1000 nmol/L, gives 29.41 nmol/L for serum folate. Converting nmol/L to ng/mL after dividing 29.41 by 2.2666 yields 12.97 ng/mL. At the two medical centers where the study reported here was carried out, serum folate levels of  $< 3.4$  ng/mL were reported as deficient and  $< 5.5$  ng/mL were considered to be insufficient.

Thiesen-Toupal et al. examined serum folate levels in outpatient setting and found that fewer than 0.06% of the samples had levels  $< 3.0$  ng/mL and about 0.26% with levels  $< 4.0$  ng/mL. The authors attributed the low prevalence of folate deficiency to the routine supplementation of food stuffs with folic acid. They recommended discontinuation of routine testing for serum folate with the caution that the results may not be generalizable to other institutions or populations [25]. This report prompted us to look at the data at two tertiary care hospitals.

## 2. Methods

The study was conducted at two medical school affiliated, tertiary care medical centers, one each in Missouri and Georgia. Both hospitals have about 500 beds each and serve as the safety net hospitals for the local population. Both hospitals are also level 1 trauma centers. The laboratories at both medical centers are accredited by the College of American Pathologists and performed the testing using Beckman analyzers and chemiluminescence methodology.

All of the tests done in one year, July 2013 through June 2014, at both medical centers, were analyzed for prevalence of various serum levels of folate in all samples and patients. All patients on whom serum folate level testing was done were included. In case of multiple results for a given patient, only the lowest value was retained. Patients below the age of 18 years were excluded from further analysis. For the patients at the Georgia medical center, the clinical parameters for the 128 patients with serum folate values below 5.5 ng/mL and an equal number of patients with highest serum folate levels,  $> 25.7$  ng/mL, were retrieved through chart review. Relevant clinical parameters between the two groups were compared by using Student's *t* test for continuous variables and Chi-square test for discrete variables. To ascertain the potential effect of location of the home address as a surrogate for economic status, zip codes for the high and low folate groups were compared. Only the zip codes with at least five members of either group were included and the data was analyzed by goodness of fit analysis. The *p* values were corrected for the number of comparisons, i.e., Bonferroni correction was applied.

The chart review was geared toward identifying risk factors for low serum folate, documentation of corrective action in patients found to have low levels, and clinical circumstances resulting in low and high serum folate levels. Other data collected were age, sex, race, bmi, hemoglobin, mean red cell corpuscular volume (MCV), and serum levels of creatinine, albumin, alanine aminotransferase (ALT), iron, ferritin, and B12. The gender distribution was nearly identical in the two groups and was not included in the statistical analysis.

To gain an understanding of the relation between serum and red blood cell folate levels, all instances of folate analysis for years 2007–2013 at the Georgia medical center were examined. In cases where serum and red cell folate levels were analyzed within 2 days of each other, the results from the two sources were compared. Reporting format for serum folate levels changed over the years. Before 2013, the highest value reported was 20.0 ng/mL and in samples with value reported as  $> 20.0$  ng/mL, the samples were arbitrarily assigned a value of 25.0 ng/mL. The serum folate equivalent of red cell folate level of 1000 nmol/L was estimated by interpolation.

IRB approval: This study was approved by the Institutional Review Boards at Georgia Regents University, Augusta, GA and University of Missouri-Kansas City and Truman Medical Center, Kansas City, MO.

## 3. Results

### 3.1. Prevalence of deficiency

The prevalence of serum folate levels in the combined sample pool, and patients, from the two medical centers is shown in Tables 1 and 2. The proportion of samples, at the two medical centers, with serum folate levels below 3.0, 3.4, 4.0, 5.5, 7.0 and 13.0 ng/mL was, 0.51%, 0.73%, 1.41%, 4.76%, 9.52%, and 40.82% respectively. The prevalence of given serum folate levels in individual patients is given in Table 2. The proportion of patients, at the two medical centers, with serum folate levels below 3.0, 3.4, 4.0, 5.5, 7.0 and 13.0 ng/mL was, 0.58%, 0.85%, 1.55%, 4.90%, 9.98%, and 43.21% respectively.

### 3.2. Documentation of replacement therapy

Medical records were examined for follow-up action in patients found to have low serum folate levels. In the 36 patients with serum folate levels  $< 3.4$  ng/mL, 16 (55.6%) had documentation of replacement. Replacement treatment was not standardized and included 1.0 mg of folic acid or multivitamin tablet without details of the composition of the multivitamin preparations. Of the total 226 patients with serum folate levels  $< 5.5$  ng/mL, 88 (38.9%) had documentation of replacement treatment (Table 3).

**Table 1**  
Distribution of serum folate levels in all samples examined in 2013–2014.

ng/mL	Serum samples			%
	GA 2696	MO 2617	Total 5313	
< 3.0	15	12	27	0.51
< 3.4	21	18	39	0.73
< 4.0	41	34	75	1.41
< 5.5	153	100	253	4.76
< 7.0	304	202	506	9.52
< 13.0	1202	967	2169	40.82

**Table 2**  
Distribution of serum folate levels in the patients examined in 2013–2014.

ng/mL	Patients			%
	GA 2075	MO 2373	Total 4448	
< 3.0	14	12	26	0.58
< 3.4	20	18	38	0.85
< 4.0	36	33	69	1.55
< 5.5	128	90	218	4.90
< 7.0	260	184	444	9.98
< 13.0	1014	908	1922	43.21

**Table 3**  
Documentation of folate supplementation in patients with subnormal folate levels.

Supplementation treatment				
ng/mL		Number	Supplemented	%
< 3.4	GA	20	10	50.00
	MO	16	10	62.50
	Total	36	20	55.56
> 3.4 < 5.5	GA	108	27	25.00
	MO	82	41	50.00
	Total	190	68	35.79
Total < 5.5		226	88	38.94

### 3.3. Comparison of demographic and health data between groups of patients with serum folate < 5.5 ng/mL and those with levels > 25.7 ng/mL

The comparative data for the two groups are given in Table 4A and B, are from the Georgia medical center. Men and women were present in nearly equal numbers in the two groups and this data was not included in the statistical analysis. There were more white patients in the group with serum folate levels > 25.7 ng/mL ( $N=78$ ) than in the group with folate levels < 5.5 ng/mL ( $N=52$ ). The difference was statistically significant by itself, but  $p$  value was > 0.05 after Bonferrone correction.

There were no significant differences between the two groups (< 5.5 ng/mL and > 25.7 ng/mL) in the values for bmi, ALT, iron, B12 and prevalence of documented excessive use of alcohol. Before Bonferrone corrections the following parameters were different between the groups at  $p$  value < 0.05: Age and MCV were higher in the > 25.7 ng/mL group; creatinine and ferritin were higher in the < 5.5 ng/mL group; more patients in the < 5.5 ng/mL group were judged to be at risk for malnutrition. After correcting the  $p$  value for the 14 comparisons, none of these parameters were significantly different between the two groups. ALT levels were significantly lower in the group with serum folate levels of < 3.4 ng/mL (Table 5).

The two groups were different both statistically as well as in a clinically meaningful way for hemoglobin, albumin and documentation of folate supplementation before collection of sample. The < 5.5 ng/mL group had lower hemoglobin (10.5 gm/dL vs. 12.02 gm/dL), and albumin (3.48 gm/dL vs. 3.96 gm/dL) as compared to the group with serum folate > 25.7 ng/mL. None of the patients in < 5.5 ng/mL group had documentation of folate supplementation before collection of sample for testing. In the > 25.7 ng/mL group 82 of 128 patients had documented administration of folate supplement as folic acid or multivitamin preparation, before sample collection. In an additional 33 patient, the subject had been at another healthcare facility immediately prior to transfer to the Georgia Medical Center or had spent a day in emergency department or another patient care unit, prior to sample collection, though documentation of folic acid administration was lacking. This difference was statistically significant as well clinically relevant.

Analysis of the distribution of patients in various zip codes did not reveal any meaningful or statistically significant difference among the patients with low and high serum folate levels (Table 6). The  $p$  value of the comparison was 0.86.

**Table 4**

A and B Comparison of clinical parameters between the low (< 5.5 ng/mL) and high (> 25.7 ng/mL) serum folate groups. The items indicated in bold were significantly different between the two groups before correction for the number of parameters compared. The items indicated in italics were statistically significantly different between the two groups after Bonferroni correction.

A										
	Age	BMI	Hb	MCV	Creat	Alb	ALT	Iron	Ferritin	B12
< 5.4 ng/mL										
Mean	53	28.5	10.5	90.1	2.01	3.48	24.96	57.75	983.15	709.8
SD	14.9	7.85	2.47	10.21	2.55	0.74	21.96	45.05	1619.55	1194.2
Number	128	125	128	128	128	125	125	67	64	128
> 25.7 ng/mL										
Mean	58.38	27.2	12.02	93.23	1.36	3.96	33.9	60.7	406.6	919.7
SD	18.17	7.1	2.44	7.74	1.62	0.57	47.6	48.1	570.5	1690.4
Number	128	128	120	120	120	118	118	41	42	124
Raw <i>p</i>	<b>0.0079</b>	0.14	<i>0.0000032</i>	<b>0.0069</b>	<b>0.017</b>	<i>0.000000079</i>	0.059	0.56	<b>0.012</b>	0.24
Corrected <i>p</i>	0.11	1.97	<i>0.000045</i>	0.096	0.24	<i>0.0000011</i>	0.83	7.91	0.17	3.31
B										
Male		Race			Alcohol		At risk		Suppl	
< 5.4 ng/mL										
56	W=52 B=71 H=2 O=3			21		107		0		
> 25.7 ng/mL										
54	W=78 B=46 H=3 O=1			28		91		82(+33)		
Raw <i>p</i>	<b>0.0083</b>			0.27		<b>0.017</b>		<i>0</i>		
Corrected <i>p</i>	0.12			3.72		0.24		<i>0</i>		

W=White, B=African American, H=Hispanic, O=other; At risk=at risk of malnutrition, Suppl=supplementation with folic acid or multivitamin.

**Table 5**

ALT values in patients with serum folate < 3.4 ng/mL vs. those with folate > 3.4 ng/mL and < 5.5 ng/mL.

Folate	< 3.4 ng/mL	> 3.4 < 5.5 ng/mL
Number	20	105
Mean	10.45	27.72
SD	2.01	23.03
<i>p</i> Value	0.000000000014	

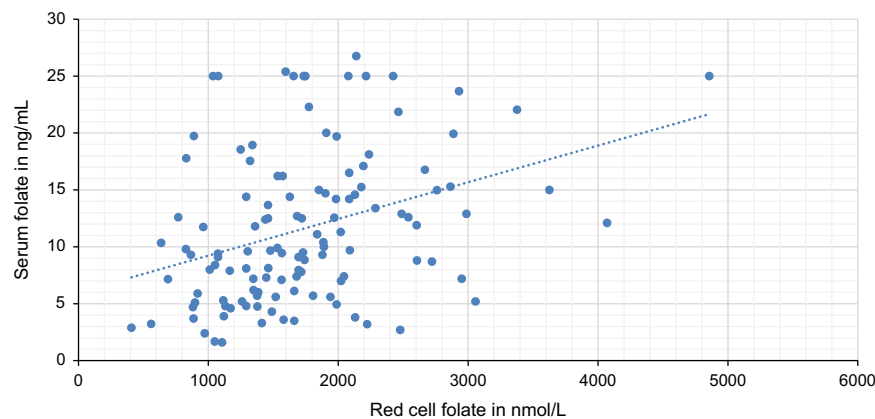
**Table 6**

Zip codes for the home addresses of the patients with low (< 5.5 ng/mL) and high (> 25.7 ng/mL) serum folate levels were compared. Only the zip codes with at least five members of one of the groups were included. The distribution of patients in the various zip codes, and by inference, economic status, were not meaningfully or statistically different. The data was analyzed for goodness of fit and *p* value was 0.86.

Zip code	Low	High
29801	5	3
30809	5	4
30815	6	6
30901	11	8
30904	12	9
30906	13	18
30907	10	5
30909	6	5

Estimation of serum folate from red cell folate measurements: RBC folate of 1000 nmol/L translated to 9.23 ng/mL of serum folate, based on the 122 samples that were comparable (Fig. 1). It is worth pointing out that the correlation between serum and red cell folate levels was, in general, poor.

One item of note was the low ALT levels in the < 3.4 ng/mL group (Table 5). Even between the two groups of patients with folate levels < 3.4 ng/mL (*N*=20) and those with folate levels > 3.4 and < 5.5 ng/mL (*N*=105), the ALT levels were highly significantly lower in the < 3.4 ng/mL group (*p* < 0.000000000014). The average value in the < 3.4 ng/mL group was 10.45 IU/L and in the > 3.4 < 5.5 ng/mL group it was 27.72 IU/L. The difference is even more remarkable in that 19 of the 20 patients in the < 3.4 ng/mL group had ALT values < 10 and were assigned values of 10 as a default. Only 4 of the 105 patients in the other group had ALT values of < 10.



**Fig. 1.** Comparison of red cell folate level in nmol/L and serum folate in ng/mL for the patients in whom both samples were tested within two days of each other. By interpolation, red cell folate of 1000 nmol/L translates to about 9.23 ng/mL of serum folate.

#### 4. Discussion

Optimum, minimum serum level of folate apparently has not been established given the wide variation in the values cited as the lower limit of normal for serum folate [2,3,9–12,14–22]. If serum folate level of 3.0 ng/mL is accepted as normal then it may well be true that folate deficiency is rare in the US and testing should be discontinued. Other sources cite serum folate levels of < 5.5 ng/mL as a state of insufficiency. It has also been stated that serum folate level of > 7.0 ng/mL would be optimal for preventing neural tube defects [17]. However, trials measuring red cell folate level and occurrence of neural tube defects reported red cell folate levels of greater than 906 nmol/L and 1000 nmol/L as being optimum for prevention of neural tube defects [9,10,17]. The highest level with the optimum protection being 1292 nmol/L of red cell folate, however, the authors suggest 1000 nmol/L as optimum [10]. Red cell folate level of 1000 nmol/L translates to about 13.0 ng/mL of serum folate as described previously, however it is not clear if the relation is linear and the extrapolation may have provided an overestimate of the serum equivalent of the RBC folate. Data of Bruyn et al. suggest that RBC folate of 1000 nmol/L would be equal to about 10.7 ng/mL [26]. Conversion of the red cell folate level of 1000 nmol/L to serum folate level in ng/mL, using the imperfect data in our patients, in whom both sources were tested, yielded a value of 9.23 ng/mL. Given that serum folate levels tend to fluctuate rapidly with folate intake status whereas red cell folate levels seem to represent the average value over the life span of red cells, using the patient data is unlikely to be an ideal way of performing the comparison. Conducting a study analyzing the serum and red cell folate levels, in healthy subjects, in a steady state intake of folate over four months may be warranted.

If we accept the WHO definition of health, “Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity”, then using a serum folate level of 3.0 ng/mL that merely prevents the appearance of megaloblastic anemia, is at best, improper [27]. Even if normalization of serum homocysteine levels were considered to be a more objective measure of folate sufficiency, the lower limit of normal serum folate levels should be set at about 7.0 ng/mL [18–20]. If we accept that preventing neural tube defects in the embryo/fetus is a physiologic function of folate, then the minimum serum folate reference level should be designated to be 13.0 ng/mL. We would support going with this high level given that no ill-effects have been documented with this level, or higher, and we recommend that optimum minimum normal level of serum folate be pegged at 13.0 ng/mL [9–12]. If the risk of exacerbating neural damage due to co-existing vitamin B12 deficiency is a consideration, as has been suggested, perhaps food supplementation should be extended to vitamin B12 as well [28,29]. Since folate deficiency in men has been suggested to be contributory to neural tube defects, this reference value should be universal, not just for women of childbearing age [30].

In addition to the well documented risk of neurologic damage due to folic acid supplementation in patients with vitamin B12 deficiency, a number of potential toxic effects resulting from high levels of folate have been recognized. Administration of folate or high serum levels of folate may not be desirable for patients on anti-folate drugs for malaria, rheumatoid arthritis, psoriasis and cancers. Folate suppresses initiation of carcinogenesis but may also act as a promoter. These and other potential undesirable effects of high intake of folate are reviewed by Smith et al. [20]. These authors suggest a serum folate level of about 26.0 ng/mL as the safe upper limit.

Based on the patient populations tested in Georgia and Missouri Medical Centers, depending on one's viewpoint about 0.5–43.21% of the patients were deficient in folate. For this discussion we will treat serum folate levels below 5.5 ng/mL as a state of folate insufficiency and accept that about 5% of the patients seen at tertiary care medical centers are folate insufficient.

The higher than expected prevalence of folate deficiency in the population tested is likely due to higher disease burden in the people-seeking healthcare at a tertiary care medical centers. In reviewing the medical records we determined that 107 of 128 patients with serum folate < 5.5 ng/mL and 91 of 128 patients with high serum folate levels were at risk of malnutrition. The risk factors were similar in the two groups and consisted of neuropsychiatric disorders, alcohol and illegal substance abuse, dysphagia, altered mental status, dementia, HIV/AIDS, advanced malignancy, gastric resection and gastric bypass surgery, end stage renal disease and major depressive disorder [31,32]. In fact, the most striking difference accounting for the high levels of serum folate in the group with levels > 25.7 was the occurrence of folic acid supplementation before collection of specimen for testing. In 82 of the 128 patients with high serum folate levels there was documentation of folate supplementation with either folic acid or multivitamins. In an additional 33 of the patients with high serum folate levels, it was likely that they received folate supplementation as medication or hospital food, as these individuals were transferred from another healthcare facility or were in the medical center during the day before specimen collection. Thus, it is likely that many, albeit an undetermined number, of the patients with high serum folate levels were folate deficient prior to supplementation. MCV was higher in the high folate group (93 in the > 25.7 ng/mL group vs. 90 in the < 5.5 ng/mL group), suggesting that the group was likely folate deficient in the recent past. In general, patients with serum folate levels < 5.5 ng/mL had higher disease burden than patient



with high serum folate as revealed by the low hemoglobin and albumin levels in the former group. At least 4.9% of the patients seeking care at our hospitals had serum folate levels in the insufficient range. It is likely and many of the patients with higher levels had received folic acid supplementation prior to testing, therefore the prevalence of folate insufficiency is higher than the data suggest.

It has been suggested that because the prevalence of folate deficiency is so low in the ambulatory persons, testing for serum should be discontinued. The same approach could also be taken for patients seeking treatment at tertiary care medical centers and all patients could be administered folic acid supplementation as that may be less expensive than testing. However, we recommend that patients continue to be tested, as serum folate levels are an indicator of malnutrition. Given that folate stores are depleted in 4–5 months of inadequate intake, and serum levels of folate decline after only a few days of negative folate balance, low serum folate level may be a leading indicator of under-nutrition of essential nutrients, obesity in the patient notwithstanding [15,16,31,32].

It was unsettling to discover that only about 39% of the patients with insufficiency of folate had documentation of supplementation with this essential vitamin. This was not an isolated event at our particular medical centers, as earlier studies reported that low serum levels of folate were acknowledged in only about half of the instances and supplementation was implemented in only a quarter of the events [15,31,32]. This could be used as an argument that patients should be administered folic acid without testing first. However doing that would miss an easily available marker of under-nutrition of essential nutrients that may require more corrective action than just administering folic acid [31,32].

This study has the usual shortcomings of a retrospective review, however, since we dealt with the real life situation of practice rather than the controlled environment of a prospective trial, the findings are expected to be generalizable.

The finding of low ALT in patients with serum folate levels of <3.4 ng/mL may be a chance occurrence despite the high statistical significance. It is plausible that low ALT is a marker of deficiency of other nutrients, such as pyridoxine that has been associated with low ALT activity. The issue warrants further investigation.

## 5. Conclusions

Serum folate levels should be tested, at admission, in tertiary care patients as this analyte serves as a leading indicator of nutritional deficiency. We recommend that normal serum folate level should be recognized to be 13.0 ng/mL.

## Conflict of interest

None of the authors have any conflict of interest to report.

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## Note added in proof

Huo et al demonstrated that combined use of anti-hypertensive medication and 0.8 mg folic acid was more effective in preventing strokes in hypertensive patients than anti-hypertensive alone. The average serum folate, at the end of the trial was 13.0 ng/mL in the un-supplemented group and 19.9 ng/mL in the supplemented group [33]. This finding further supports the concept of establishing serum folate level of 13.0 ng/mL as the lower limit of normal value.

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